

Immunoglobulin and complement in normal skin

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SUMMARY Cryostat sections of normal skin from 57 white adults were examined by direct and indirect immunofluorescence for immunoglobulins, complement factors, and transferrin. The results for basement membrane zone (BMZ) were significantly different for the 11 face and 46 non-face biopsies: in the face, IgM was found in five, IgG in two, IgA in one, and C3 in none, whereas, in non-face, IgM was present in six, IgG in none, IgA in one, and C3 in five.

The results for dermal vessel walls (DV) were not apparently different for face and non-face; in the 57 biopsies IgM was present in one, IgG in none, IgA in one, and C3 in one.

The 11 biopsies from the face and 26 of the non-face biopsies were examined further. No IgD or C4 was identified, but one case (scalp) showed BMZ C1q, properdin, and transferrin, and in two cases (one face, one non-face) DV properdin was found.

Cytoid bodies (IgM and IgA) were present in moderate numbers in one case; all other positive reactions were finely granular.

Studies of extracellular immunoglobulin and complement in frozen sections of skin were originally found to be helpful in the diagnosis of bullous diseases and lupus erythematosus but now contribute to diagnosis, prognosis, or management in a wide range of skin diseases (Tuffanelli, 1975). There have been few studies of normal skin; in the two largest series no immunoglobulin or complement was found in 65 normals (Schroeter *et al.*, 1976), and IgM was found in 15/23 normals (and IgG in 7/23, IgA in 0/23) (Baart de la Faille-Kuyper *et al.*, 1974). In the present study, normal skin from 57 adults was examined for the standard IgG, IgA, IgM, and C3; in addition, 37 cases were examined for other complement stages (C1q and C4), properdin, transferrin, and IgD. C1q and C4 are found only in classical complement activation, whereas C3 (the main amplification step) is found in both classical and alternate pathway activation; properdin forms part of the activation sequence for the alternate pathway and is usually considered to indicate involvement of the alternate pathway—which, however, is also activated by a feedback loop in the classical pathway (Roitt, 1977); properdin has been described in affected and unaffected skin of patients with systemic lupus erythematosus (Schrager and Rothfield, 1976; Schroeter *et al.*, 1976). Transferrin indicates non-specific leakage of protein through vessel walls as it is neither an immunoglobulin nor involved in the

complement sequence. IgD was included because it has been described in dermatitis herpetiformis and pemphigoid (Cormane and Giannetti, 1971) and in cutaneous vasculitis (Weidner, 1975).

Material and methods

Skin biopsies were taken from 57 white adults at the time of removal of a known lesion considered to have no association with generalised skin abnormalities. The skin was embedded in OCT (Lab-Tek, Miles), frozen in liquid nitrogen, and stored at -70°C for a few days before cryostat sections were cut at $5\text{ }\mu\text{m}$. Eight sections were examined from each biopsy initially—two each for IgA, IgG, IgM, and C3—using the direct fluorescence technique, with optimally diluted conjugates of sheep anti-human IgG, IgM, and IgA (Wellcome) and rabbit anti-human C3 (Dakopatts). Examination for fluorescence was Ploem incident illumination (Leitz), with exciter filter $2 \times \text{KP } 490$, dichroic mirror TK 510, suppression filter K 515, and edge filter GG 475.

In all cases normal skin was taken as far as possible from the biopsy of the lesion. In 11 cases the lesion was a sebaceous cyst (9 negative and 2 positive immunofluorescence), 11 inflammatory foci (1 positive), 9 basal cell carcinomas (2 positive), 5 benign pigmented naevi (1 positive), 5 dermatofibromas (3 positive), 4 senile keratoses (1 positive), 3 basal cell papillomas (none positive), 2 pilomatrixomas (1 positive), 2 invasive squamous car-

cinomas (1 positive), 2 hidradenomas (1 positive), 1 haemangioma (positive), 1 pyogenic granuloma (positive), and 1 Bowen's (positive).

Eleven biopsies were from the face, and basement membrane zone IgM was found significantly more frequently in the face (5/11) than in non-face (6/46). All biopsies from the face (11), nine of the 11 non-face biopsies with positive findings (satisfactory sections could not be obtained on the other two), and 17 non-face biopsies with negative results were therefore re-examined for IgG, IgA, IgM, and C3 and further examined by indirect immunofluorescence using goat antisera to human C1q, C4, transferrin, and properdin (Flow Laboratories), and to IgD (Nordic Diagnostics), with fluorescein-labelled rabbit antiserum to goat IgG (Miles-Yeda Ltd).

Results

All basement membrane zone (BMZ) and dermal vessel wall (DV) reactions were finely granular (Figs 1 and 2). The total results are given in the Table.

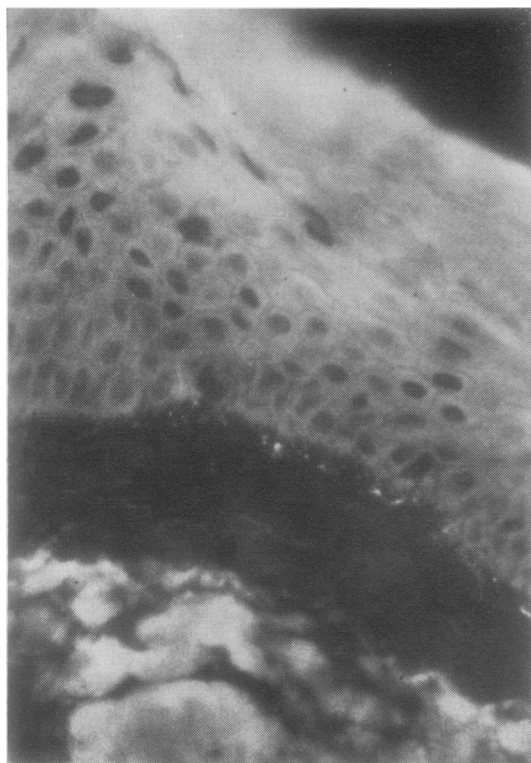


Fig. 1 Basement membrane zone IgM. Immunofluorescence $\times 480$.

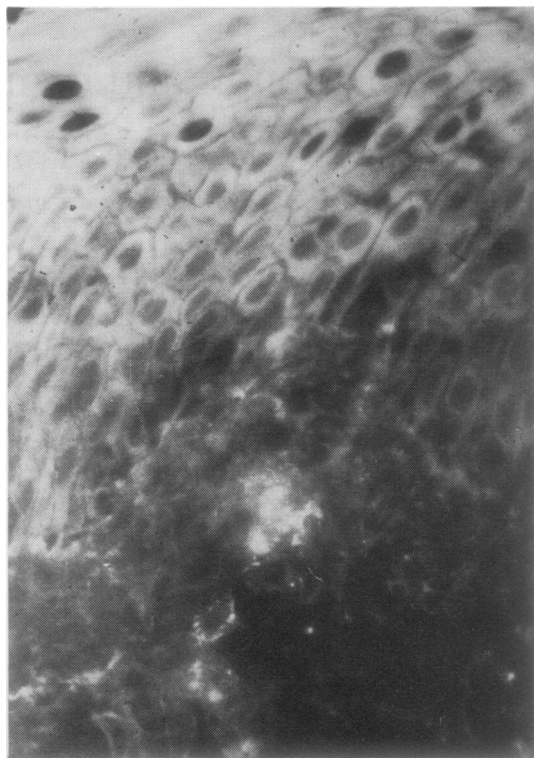


Fig. 2 C3 in papillary dermal vessel wall. Immunofluorescence $\times 480$.

Cytoid bodies (IgM and IgA positive) were present in moderate numbers in one case (non-face), which also showed BMZ C3.

FIRST ASSESSMENT (IgG, IgA, IgM, and C3)

Face

Five cases had BMZ IgM; one of these also had BMZ IgG, and one also had BMZ IgG and BMZ IgA. The case with BMZ IgM and IgG had telangiectasia. Six cases gave no positive results.

Non-face

Six cases had BMZ IgM; one of these also had BMZ IgA and BMZ C3. Four cases had BMZ C3 only. One case had DV IgM and C3. Thirty-five cases gave no positive results.

SECOND ASSESSMENT (IgG, IgA, IgM, and C3)

Face

The six negative cases and the three cases with BMZ IgM only remained the same. The case with BMZ

Table Summary of results (total tested was 57 for IgM, IgG, IgA, and C3, and 37 for C1q, C4, properdin, IgD, and transferrin)

	IgM	IgG	IgA	C3	C1q	C4	Properdin	IgD	Transferrin
Basement membrane zone									
11 face	5	2	1	0	0	0	0	0	0
46 non-face	6	0	1	5					
26 non-face					1	0	1	0	1
Total	11	2	2	5	1	0	1	0	1
Dermal vessel walls									
11 face	0	0	1	0	0	0	1	0	0
46 non-face	1	0	0	1					
26 non-face					0	0	1	0	0
Total	1	0	1	1	0	0	2	0	0

IgG and IgM on the first test became BMZ IgM positive only. The case with BMZ IgM, IgG, and IgA on the first test became BMZ IgM and DV IgA positive.

Non-face

The 17 negative cases and the five cases with BMZ IgM only remained the same. Of the four cases with BMZ C3 on the first test, two became negative, and satisfactory sections could not be obtained from the other two. The case with BMZ IgM, IgA, and C3 became BMZ IgA positive only. The case with DV IgM and C3 became negative.

SECOND ASSESSMENT (C1q, C4, properdin, transferrin, and IgD)

None of the 37 cases (11 face, 26 non-face) tested for C4 and IgD was positive. Properdin was found in the dermal vessel walls in two of the 37 cases (one face and one non-face) and in the BMZ in one case; this biopsy also showed BMZ C1q and BMZ transferrin. The other 36 showed neither.

Discussion

The difference between the two assessments of IgG, IgA, IgM, and C3 may be due partly to sampling error, as is recognised in dermatitis herpetiformis (Jabłońska *et al.*, 1973), but as a trend towards negative results is shown by the second assessment, part of the difference is probably due to deterioration of the tissue with storage. The interval between the two tests was 1-11 months for the whole group and 2-11 months for the cases which changed; storage was at -70°C . Storage is known to affect reactivity (Beutner *et al.*, 1973), although under optimal conditions (-70°C) reactivity is thought to remain stable for at least three months.

The single biopsy with BMZ properdin, transferrin, and C1q was from the scalp in a patient with a sebaceous cyst. The other scalp biopsy in the series gave negative results throughout.

The single case with typical cytooid bodies had no clinical or histological evidence of skin disease, though typical cytooid bodies have not previously been described in normal skin. Cytooid bodies are a prominent feature of lichen planus but also occur in lupus erythematosus, eczema, and occasionally in other skin diseases (Abell *et al.*, 1975).

Schroeter *et al.* (1976) examined skin biopsies from the leg of 65 controls (15 with no skin abnormality and 50 with skin diseases other than vasculitis) for IgG, IgA, IgM, C1q, C3, factor B, properdin, and fibrin and found no positive results. Baart de la Faille-Kuyper *et al.* (1974) examined skin biopsies from the extensor surface of the forearm of 12 female and 11 male healthy volunteers and found papillary vessel wall IgM in 15 (with BMZ IgM in five); vessel wall IgG was found (with albumin) in seven cases (with BMZ IgG in three); vessel wall C4 was found in four cases, and C5 in 10. The BMZ findings are not markedly dissimilar from those in the present study, but the high frequency of dermal vessel immunoglobulin and complement is unlike any other published work, and the presence of albumin also casts doubt on the significance of the dermal vessel results.

BMZ IgG is found in lupus erythematosus, typically as a sharply defined linear or coarsely granular band; a weak, poorly defined, fibrillar band is present in some cases of facial dermatosis, especially rosacea and telangiectasia (Burnham and Fine, 1971; Abell *et al.*, 1974), and although this is usually IgG (23/37 cases of rosacea and telangiectasia), IgA (11/34) and IgM (3/14) are also found (Jabłońska *et al.*, 1970). None of these patterns was found in the present study.

Although experimentally in the Arthus reaction complexes have completely disappeared from the lesions by 18 hours (Cream *et al.*, 1971), in man immunoglobulin and/or complement can frequently be identified in the lesions of vasculitis—IgG, IgM, and C3 (Schroeter *et al.*, 1971), IgM, IgA, and C3 (Sams *et al.*, 1975), and IgD, IgA, IgG, C3, and C5

(Weidner, 1975). In the present study DV IgM was present in one case (with C3) and DV IgA in another; properdin was found in two cases, and IgG and IgD in none.

The results in the present study suggest that immunoglobulins and complement are seldom present in normal dermal vessel walls, that BMZ IgM is commonly present in normal skin (especially in the face), and that BMZ IgG, IgA, and C3 are occasionally present in normal skin. The pattern of fluorescence is often helpful in distinguishing normal from pathological, as in the distinct IgG band in lupus erythematosus and granular IgA in the papillary dermis in dermatitis herpetiformis.

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